Experimental Researches on Vegetable Assimilation and Respiration. XIII.*—*The Development of Photosynthetic Activity during Germination.*

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Introduction.

We must assume that the development of every leaf begins with a stage in which it is incapable of performing the function of assimilation. It is a matter for investigation to determine whether this stage is passed when the tiny leaf first becomes visible in the bud or whether it persists even after the leaf is unfolded and fully green.

For seedlings this matter is very important biologically, because it determines when the plant can begin to make additions to its dry weight and start its natural development. Analysis of results of growth experiments on such plants as maize indicates that the early assimilatory power is very feeble.†

In most methods of experimenting on the relation of photosynthetic activity to the development of chlorophyll-greenness the work is complicated by the fact that during the period of illumination of the experiment the chlorophyll continues to increase in amount so that one cannot maintain a constant greenness for precise investigation. A method of experimentation devised by Dr. F. F. Blackman, and used in this laboratory, allows photosynthesis to be measured in an atmosphere with so low an oxygen pressure that further chlorophyll development is inhibited, and the green tone can be kept constant for days while the leaf is exposed to light.

With this procedure the development of photosynthesis has been followed from about zero to nearly full activity. The results, besides throwing light on the nature of the photosynthetic mechanism, explain why Willstätter failed to confirm the results obtained by Irving* in this laboratory in 1910.

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* Since the publication of Part IX ('Roy. Soc. Proc.,' B, vol. 83, p. 389) of this series, carried out in the Botany School, Cambridge, under the general direction of Dr. F. F. Blackman, the following parts have appeared elsewhere:—X. A. A. Irving, "The Effect of Chloroform upon Respiration and Assimilation," 'Annals of Botany,' vol. 25, p. 1077 (1911). XI. D. Thoday, "On the Effect of Chloroform on the Respiratory Exchanges of Leaves," 'Annals of Botany,' vol. 27, p. 697 (1913). XII. A. M. Smith, "The Temperature-Coefficient of Photosynthesis," 'Annals of Botany,' vol. 33, p. 517 (1919).

† Briggs, G. E., Kidd, F., and West, C., "Quantitative Analysis of Plant Growth," 'Ann. Appl. Biol.,' vol. 7, No. 1 (1920).

At this point I wish to express my thanks to Dr. F. F. Blackman for suggesting the research and for advice and criticism.

SECTION I.—*The Relation of Photosynthetic Activity to Age and Chlorophyll Development in Young Leaves.*

Method and Procedure.—The principle of Dr. Blackman's new method for measuring the output of oxygen during photosynthesis is to illuminate the leaf in a small closed chamber in an atmosphere of hydrogen with added carbon dioxide. The oxygen produced is determined by circulating the atmosphere over palladium black, when the oxygen unites with twice its own volume of hydrogen. The resulting reduction in gas volume is measured by means of a gas burette in connection with the apparatus. As this reduction is threefold that of the oxygen produced, very small amounts of assimilation can be accurately measured. The volume of the synthetic water is of course negligible.

The illumination was supplied by an ordinary incandescent filament lamp of thirty-two candle power, and was varied for certain test experiments by changing the distance of the lamp from the chamber.

The percentage of carbon dioxide in the apparatus was about five, and was never allowed to become so small as to limit the rate of photosynthesis.

Various materials, including the young buds of the bean (*Vicia Faba*), the young leaves of the oat (*Avena sativa*), and of the dwarf French bean (*Phaseolus vulgaris*), were used in the preliminary experiments, but the last-mentioned was found to be the most suitable, and was used for the more critical experiments; in *Phaseolus* the leaf-area did not change during the experiments, whilst in the case of the others, slight unfolding of the leaves took place.

Prior to sowing, the seeds were soaked for a short time in a small quantity of water at the temperature of the laboratory (12°–15° C.). When the seeds were swollen they were sown in soil in pots and germinated in a hot box (24°–25° C.) protected from the action of light with a black cloth. For the experiments young yellow leaves were cut from these etiolated plants and exposed to light until they had acquired the desired tone of greenness. The light was usually that of an incandescent filament lamp in a dark room, and was of moderate intensity, so that no retardation of greening was caused by too strong or too weak an illumination. The leaves, when sufficiently greened, were transferred to the apparatus, and their photosynthetic activity measured by their output of oxygen. Except where otherwise stated, they were transferred to the apparatus immediately after exposure to light. Between the periods during which the assimilation was measured, the chamber was disconnected from the apparatus, the hydrogen replaced by air, and the chamber, with the leaves still in it, placed in the dark. In this way the leaf was kept at one tone of greenness for many days.

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* Irving, A. A., "The Beginning of Photosynthesis and the Development of Chlorophyll," 'Annals of Botany,' vol. 24, pp. 805–818 (1910).

At the completion of the experiment each set of leaves was dried at 100° C. until no further loss of weight was observed.

Results.—The details of the experiments on *Phaseolus* set out in the Appendix are summarised in Table A below.

The assimilatory power is expressed as the output of oxygen in cubic centimetres per gramme dry-weight of leaf per hour. The volume is reduced to 0° C. and 760 mm. of mercury. It is realised that this is a measure of "apparent" and not of "real" assimilation, since oxygen consumption, due to respiration, is neglected, and consequently, when the assimilatory power is given as x c.cs., a value of about 1·3 c.cs., as shown by the respiration experiments, should be added. The "apparent" assimilation, however, is suitable for the comparative purposes of these experiments, and to substantiate the conclusions drawn.

Table A.—*Phaseolus vulgaris.* Assimilatory Power of Young Seedling Leaves of different Ages and Degrees of Greenness as Measured by Oxygen Production.

Day from sowing.	Assimilatory power.				
	Expt. I.	Expt. IA.	Expt. II.	Expt. IIA.	Expt. VI.
0–7th	Grown	in dark.			
8th	Greened by li	ghting in air.			
9th	1·3	In dark.			
10th	2·5	4·1			
11th	In dark	5·5	—	—	Greened by lighting in air.
12th	7·0	6·7	—	—	" "
13th	7·0	—	Greened by li	ghting in air.	" "
14th	—	—	7·8	In dark	" "
15th	—	—	9·5	"	" "
16th	—	—	10·0	10·5	" "
17th	—	—	—	—	12·0

Table A—*continued.*

Day from sowing.	Assimilatory power.				
	Expt. III.	Expt. IIIA.	Expt. IV.	Expt. IVA.	Expt. V.
0–7th	Grown in dark.		Grown in dark.		
8th	Greened by lighting in air.				
9th	0	In dark.			
10th	5·0	”			
11th	10·4	”			
12th	—	9·2	Greened by lighting in air.		
13th	—	—	6·6	In dark.	
14th	—	—	7·8	”	
15th	—	—	9·1	7·3	
16th	—	—	—	7·0	Greened on plant.
17th	—	—	—	—	” ”
18th	—	—	—	—	” ”
19th	—	—	—	—	” ”
20th	—	—	—	—	” 12·0 ”

NOTE.—In Expts. I, IA, II, IIA, IV, and IVA, the period of greening was approximately the same, and likewise the tone of greenness (“ green-yellow ”) developed.

The leaves of Expts. III and IIIA were greened for a longer period, and the tone of green obtained was deeper (“ yellow-green ”). In Expts. V and VI the leaves were full normal green and are included for comparative purposes.

The conditions during illumination were the same in all experiments. Temperature 11°–16° C.

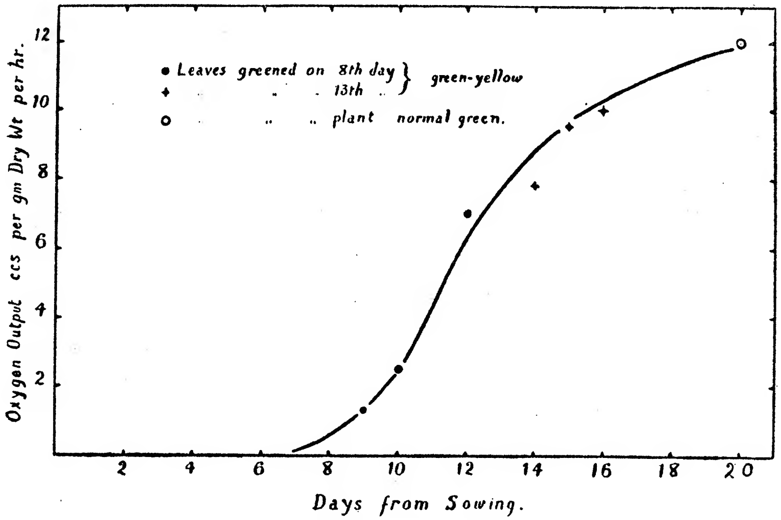
The value given for the assimilatory power is the average for the period during which the leaves were illuminated in the apparatus—a preliminary of about one hour being allowed in each case. For details see Appendix.

The first noticeable feature of the results recorded is that the assimilatory power of leaves, which have developed a very small portion of their normal content of green pigment, is a quantity which depends more upon the age of the leaf than upon its colour. The leaves of Expts. I and II are comparable in that they are from plants which were sown together. The main difference is that the leaves of Expt. I were exposed to light on the eighth day from sowing, whilst those of Expt. II were five days older when exposed. The assimilation of the former on the first day after greening was only 1·3 units, whilst that of the older leaves was 7·8. Still more striking is a comparison of Expts. III and IV. The younger leaves of Expt. III produced no surplus oxygen during the seven hours they were in the apparatus. These leaves had been exposed to light for 25 hours, and during that time had developed a yellow-green colour. On the other hand, those of Expt. IV, which were four days older, showed an output of 6·6 units of oxygen, although they had been exposed to light only 13 hours, during which time they had become only green-yellow.

The second feature of note is that, whatever the amount of the oxygen output on the first day after greening, it increased during the following days until it reached a value, in some cases, almost as large as that of leaves which had been exposed for three or more days, and had attained a normal green colour. Reference to Expt. III will reveal that leaves, which on the first day showed no assimilation, on the third day had reached a value of 10·4, whilst the normal leaves of Expts. V and VI under similar conditions gave a value of 12 units. Yet throughout each increasing series there was no increase in greenness of the material.

Moreover, if the leaves after greening are kept in the dark for three days, they show at the end of that period an assimilatory power of the same dimensions as that of similar leaves, which have been exposed to light in the apparatus during a considerable part of the three days. For example, the leaves of Expt. IIIA received the same treatment as those of Expt. III, except that they were in the dark whilst the latter were in the apparatus. At the end of three days, however, they showed an assimilation of 9·2 units as compared with the 10·4 of Expt. III—values almost the same.

That the above features are general for such partially greened etiolated leaves is borne out by numerous results obtained with other material. Additional evidence from *Avena* and *Vicia* is set out in the Appendix.



The results of Expts. I and II, comparable in that the leaves were of similar tones of greenness, are presented graphically in the accompanying figure, where the abscissæ are days counted from sowing and the ordinates oxygen output in c.cs. per grm. dry-weight per hour. The value for the fully green leaves of Expt. VI is included for comparative purposes.

Seeking the interpretation of these phenomena we may dispose of certain factors to which the increase of assimilation, with progress of time after greening, might have been attributed in the first instance. These are (1) increase in chlorophyll-content during that time; (2) increase in leaf area; (3) fall in respiration, since the assimilation measured is the "apparent" not the "real" assimilation; or, possibly (4) a combination of these factors.

It seems most improbable that the increase is due to increased chlorophyll-content, since the same increase in assimilation was observed when the leaves were kept in the dark. To gain definite information on this point one sample of leaves was taken directly after greening, and another sample of the same lot after exposure to light in the apparatus; both samples were dried and extracted with 85 per cent. acetone, in such quantities that the ratio of dried leaf material to extract was the same in both cases. These extracts were indistinguishable in tone of greenness, thus showing that the conditions of the experiment were really such as to inhibit further development of chlorophyll.

The change in size of the leaves during the experiments was found to be negligible.

Knowing that the respiration of young leaves is relatively large, it was decided to determine its magnitude for material similar to that used in the assimilation experiments, and to measure the falling respiration during a three-day period.

To estimate the respiration, about twenty young etiolated leaves of six or seven days from sowing were placed in an air-tight flask containing ordinary air; the flask was placed in the dark, and at intervals samples of gas were withdrawn and analysed for carbon dioxide, and at the same time the gas in the flask was replaced by ordinary air. The results are recorded in Table XII in the Appendix, the respiration being expressed as cubic centimetres of carbon dioxide per gramme dry weight of leaf per hour.

The first value, 1·4, is most probably too high, as it represents the rate of respiration during the five hours subsequent to cutting: respiration is usually greatly increased by wounding and handling. If it be assumed that the respiration of these leaves decreased from 1·3 to 0·8 units during the three days, then the magnitude of the fall in respiration is not likely to be underestimated. Consequently the fall in respiration cannot be held to account for a rise in "apparent" assimilation of more than 0·5 units, unless the coefficients of gaseous exchange depart very widely from unity. Moreover, this fall in respiration is due chiefly to starvation, that is, disappearance of respirable material. In the case of the leaves used in the experiments on photosynthesis, the loss of material by respiration was more than made good by assimilation. It must, therefore, be concluded that the extent of the increase in "apparent" assimilation that could be attributed to decrease in respiration is most probably less than 0·5 units. In all cases, the increase of assimilation was of a considerably greater magnitude.

Evidence that the leaves respired normally in the photosynthetic apparatus in the light is afforded by the fact that they appeared quite normal at the end of the experiments, while some younger leaves which were used, but which failed to put out sufficient oxygen, died as a result of the anærobic conditions. These injured leaves developed the characteristic odour of the pod of the French bean, an odour absent from the normal leaf.

From the above consideration, it would appear that the inevitable conclusion to be drawn is that this increase in assimilation with progress of time cannot be due to the factors just examined, but must be attributed to increase in some "internal" factor other than chlorophyll. This aspect of the matter will be further analysed in the next section.

SECTION II.—*The Bearing of these Results on the Conception of the Photosynthetic Mechanism.*

If the only data available were those given in Table A, it might be assumed that some "protoplasmic" factor was developing from day to day and throughout acting as a limiting factor.

Before considering the question further, it will be well to be clear as to what is meant by a "limiting factor." A factor is said to be limiting a reaction when an increase in that factor results in an increase in the rate of the reaction. To take the case of chlorophyll and assimilation, chlorophyll is said to be limiting assimilation when an increase in chlorophyll leads to an increase in the rate of assimilation. It is conceivable, however, and even probable that an amount of chlorophyll-content which would be limiting under conditions of weak illumination would no longer be limiting under conditions of stronger illumination.

In considering a process such as photosynthesis, it is perhaps better to think of the reaction as consisting of stages such as diffusion, photochemical, and "dark" or chemical stages. Each of these stages conceivably depends upon two or more factors. For example, it is possible to think of the photochemical stage as dependent upon light intensity and chlorophyll-content. This stage then is limiting when an increase in either of these factors leads to an increase in the rate of assimilation. Similarly, the chemical stage may depend upon protoplasmic development and upon temperature, and consequently a degree of protoplasmic development which is limiting at a lower temperature, may cease to be limiting at a higher temperature. Further, a factor such as temperature may affect more than one stage of the process.

This analysis of the process into stages emphasises the importance of due consideration being given to such conditions as illumination and temperature before making the statement that internal factors, such as chlorophyll or those of a protoplasmic nature, are limiting. Such considerations are not always given.

On the other hand, if, under constant conditions of illumination and temperature, assimilation increases without a corresponding increase in an internal factor, such as chlorophyll, then that factor is not limiting the rate of reaction under these conditions. Such a conclusion has been arrived at with regard to chlorophyll as a result of the considerations set forth in Section I.

To elucidate further the nature of the limiting factor in the young leaves, the effect of reduced illumination on the rate of assimilation was tested. This was done on six different dates by increasing the distance of the lamp from the chamber from 6 cm. to 12 cm. The results are presented in Table B.

Table B.—Effect of Reduction of Illumination on Photosynthetic Activity of incompletely Greened Young Leaves of *Phaseolus*.

Expt. No.	Day from sowing.	Assimilation in cubic centimetres of oxygen.		Temperature.
		Lamp at 12 cm.	Lamp at 6 cm.	
III	9th	—	0	
	10th	3·3	5·0	12°–13° C.
	11th	4·3	10·4	13°–14·5° C.
IIIA	12th	3·9	9·2	14°–15° C.
IV	13th	3·3	6·6	12·5–14° C.
	14th	3·7	7·8	12° C.
VI	17th	5·0	12·0	14·5°–15·5° C.
Leaves nor	mal green.			

The exact ratio of the assimilation of the two different light intensities is of no great significance for our analysis. It was proved by a number of experiments that with fully-greened leaves the temperature, when about 15° C., was limiting when the lamp was 6 cm. distant, and consequently the assimilation values given for the fully active leaves under these conditions of illumination will be dependent upon the temperature when the latter is at or below 15° C.

If the assumption that the "internal" factor which is limiting the photosynthetic process in the early stages is purely of a protoplasmic nature, that is, one involved in the chemical or dark stage of the process, be correct, then as the following consideration shows, reduction of illumination should not have the effect observed.

The maximum value of the assimilation for the fully-greened leaves, with the lamp 12 cm. distant, is 5. The assimilation is limited to this value in this case by light, that is, by the photochemical stage, since an increase of the intensity of illumination leads to an increase in assimilation. If the assimilation in the early stages of the young leaves were limited by a "protoplasmic" factor only, then whenever this factor determined the assimilation of five or more units when the lamp was 6 cm. distant, the assimilation should not be reduced below the value of five when the lamp is removed to 12 cm., since the photochemical part of the mechanism is assumed to allow of this value. As this is not the case, the internal limiting factor, which determines the assimilation in the early stages of the young leaves when the lamp is 12 cm. distant, must be concerned with the photochemical part of the mechanism.

To consider the question from another point of view, in all cases with the lamp 12 cm. distant the assimilation is limited by the photochemical stage, since an increase in the intensity of illumination results in an increase of assimilation, and consequently as the earlier stages give a smaller value than that given by the fully active leaves under the same conditions of illumination, we must conclude that this smaller value is to be attributed to an internal factor concerned with the photochemical part of the photosynthetic mechanism. This limiting factor increases its potentialities from day to day, as shown by the increasing values of the assimilation when the lamp is maintained at 12 cm.

The question as to whether the smaller values of assimilation in the earlier stages under the conditions of higher illumination were due to this same limiting factor could have been decided by testing whether these smaller values could have been increased by increasing the intensity of the illumination still further. Had the limiting factor been purely of a photochemical nature the assimilation would have increased with increasing intensity of illumination until the maximum of about 12 units, determined by the chemical stage, had been reached. The light intensity at our disposal was not sufficiently great to decide this question.

Speculation as to the nature of this factor is very attractive, but before it can be done with advantage more knowledge is required. With certainty it can be said that, although it is involved in the photochemical stage, it is not chlorophyll alone, since it increases in value without increase in chlorophyll-content.* Further, the degree of development of this factor is primarily dependent upon age and time after greening, as shown in Section I. Consequently the indications seem to be that this factor depends upon: (1) chlorophyll, since without chlorophyll the photochemical part of the photosynthetic mechanism is absent, or practically so; (2) some other factor, working in correlation with chlorophyll, which factor is dependent upon age, as very young leaves, no matter what their chlorophyll-content, cannot function as assimilating organs (see Table VII in Appendix). Further evidence as to its nature is not sufficiently definite to warrant more discussion.

SECTION III.—*A Consideration of the Results obtained by Irving and by Willstätter on the Relation of Greening to Photosynthesis, and an Explanation of the Apparent Contradiction.*

Irving, in 1910, working with F. F. Blackman, came to the conclusion that the development of photosynthetic activity in the young leaves of seedlings lagged behind greening. In other words, as the present writer's experiments show, the development of photosynthetic activity is dependent upon some factor other than chlorophyll. Irving's experiments were carried out with a small carbon dioxide supply, namely, that of respiration. She found that, under these conditions, the young leaves were unable to reduce measurably their carbon dioxide output until after they had reached an advanced stage of greening. The illumination used was greater than that required by normal leaves to utilise the whole of their respiratory carbon dioxide.

Willstätter and Stoll,† in 1915 working under what appeared to be similar conditions, found that leaves, which had developed only a very small portion of their normal chlorophyll-content, were able to utilise for assimilation the whole of their respiratory carbon dioxide. This result being in direct contradiction to that obtained by Irving, they sought, without success, for some explanation.

The only difference of procedure which they consider is, that in their experiments they took the temperature of the leaves, and thus were able to avoid an increased leaf-temperature on illumination, whilst Irving took the temperature of the bath in which the leaf-chamber was placed, and consequently the leaf-temperature was increased on illumination. Willstätter has shown in other experiments that leaves give up carbon dioxide when the temperature is raised. This, however, continues for about an hour only. A consideration of the small amount of carbon dioxide in the air current, the small change of temperature, and the duration of Irving's experiments, shows that this source of error is negligible. Willstätter also agrees that this detail in Irving's method of experimentation cannot be the cause of her results being different from his own.*

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* It may be as well to note that there is no definite evidence as to the nature of the green pigment in young leaves, as Willstätter's measurements of chlorophyll are colorimetric not chemical, whilst other writers refer to such substances as proto-chlorophyll. In this paper the term chlorophyll is used for the green pigment, whatever its nature.

† Willstätter, R., and Stoll, A., "Untersuchungen über die Assimilation der Kohlensäure," 'Ber. Deut. Chem. Ges.,' vol. 48, pp. 1540–1564 (1915).

Under conditions of more concentrated carbon dioxide supply, he found that, with increasing chlorophyll-content, the leaves showed increasing assimilation. The following Table is an extract from his results obtained with *Phaseolus*:—

Table C.—Experiments of Willstätter and Stoll on Photosynthesis with Etiolated *Phaseolus.*

Carbon dioxide 5 per cent ; illumination 48,000 lux; temperature 25° C.

Exposure to light for greening.	Colour of leaves.	Chlorophyll per 10 grm. fresh weight.	Assimilation CO_2 per 10 grm. fresh weight per hour.	Assimilation number.
		mgrm.	grms.	
—	Yellow	<0·1	0·014	>70
Six hours…………	Green-yellow	0·6	0·080	133
Two days ………	Yellow-green	8·0	0·172	24
Four days ………	Grass green	15·6	0·204	13·3
Grown in light;	normal green	18·6	0·174	9·4

Jørgensen and Stiles,† in their review of the literature on carbon assimilation, agree with Willstätter that the latter's results show, contrary to Irving's conclusion, that, during the development of chlorophyll, it is this factor which is limiting the assimilation. They suggest that possibly Irving's results were due to light being limiting.

The only grounds they give for the conclusion that Willstätter has shown chlorophyll to be the limiting factor is the fact that the partially greened leaves have high assimilation numbers, that is, high compared with normal leaves. Consideration, however, indicates that the only conclusion to be drawn is that, with increasing chlorophyll-content, the assimilation does not increase proportionately; constancy of assimilation number, with rising chlorophyll-content, would be a nearer indication of chlorophyll being limiting. In no way can it be said that Willstätter's results prove chlorophyll to be limiting in the early stages. The increase in assimilation, with increase in chlorophyll-content observed, may be due to increase in other internal factors uncontrolled and not brought into account. This point will be taken up again later.

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* Willstätter, R., and Stoll, A., 'Untersuchungen über die Assimilation der Kohlensäure,' Berlin, 1918, p. 131.

† Jørgensen, I., and Stiles, W., "Carbon Assimilation," 'New Phytologist,' vol. 15, p. 184 (1916).

To return to Irving's experiments, and the suggestion that here light was the factor limiting the assimilation, there would appear to be no grounds for this suggestion. Jørgensen and Stiles state that Irving exposed her plants to the feeble light of a north window. Reference to her paper, however, will show that such was the case in her two preliminary experiments, but that in her critical ones she used two Keith high-pressure burners. Further, since she obtained the same results with feeble light as with strong light with leaves which had developed an almost normal amount of chlorophyll, it may be concluded that the intensity of illumination had no great effect on the results.

There still remains the task of explaining the difference between the results obtained by Irving and by Willstätter under similar conditions. At first, it seemed possible that the difference might be due to the fact that, except in her first experiment, Irving's leaves greened after being cut from the plant, and that they greened in an atmosphere where the carbon dioxide was removed as soon as formed. A comparison of leaves, which had developed their chlorophyll while attached to the plant and in ordinary air, with those which were cut and exposed to light in an atmosphere deprived of carbon dioxide, showed that there was no essential difference between their rates of assimilation.

In Willstätter's earlier account of his work he gave no record of the history of his leaves, but in his 1918 publication he states that his experiment, which was done under similar conditions to those of Irving, was performed with *Phaseolus* leaves which were exposed for six hours on the 14th day from sowing, when the shoots were about 12 inches high, and that the assimilation was measured on the following day. Irving's material, however, was taken when the shoots were only 4 to 5 inches long, that is, five or six days from sowing. Further, Irving measured the assimilation directly as the leaves greened; not 24 hours afterwards. The writer's experiments show that age and lapse of time after greening are, however, just the most important factors, and, consequently, it seems safe to conclude that the discrepant results are due simply to this, that Irving worked with very young leaves while Willstätter used distinctly older material. The experiments recorded in this paper pass from the early phase where there is distinct chlorophyll and no assimilation to the later phase where, with still little chlorophyll, the photosynthesis is quite large.

Irving's experiments also show that the assimilatory power goes on developing, though further greening is arrested by darkness, a point demonstrated by the writer's experiments. For example, in her second experiment, although, after having developed a considerable tint of green, the leaves showed no reduction of respiratory carbon dioxide, yet after the lapse of 18 hours, though further greening was inhibited by darkening overnight, they showed definite signs of photosynthesis on lighting again.

The Photosynthesis of Young Leaves Developing on Mature Plants.

A consideration of Willstätter's work on developing leaves of trees in spring and on the leaves of golden varieties very poor in chlorophyll adds further support to the conclusions drawn above.

Table D.—Relation of Photosynthesis to Amount of Chlorophyll in Different Types of Leaves of *Quercus Robur* (Willstätter and Stoll). Conditions of experimentation the same as in Table C.

Date.	Description.	Chlorophyll-content.	Assimilation.	Assimilation number.
May 2	Normal young	6·6	0·072	10·9
„ 20	„ „	8·6	0·136	15·8
June 9..............	„ older	21·6	0·194	9·0
July 20	„ „	25·0	0·196	7·8
„ 18	Golden variety	1·9	0·103	55·0

Note.—Chlorophyll-content and assimilation are given in same units as in Table C.

In considering these results it will be as well to bear in mind the conclusions arrived at previously as to the interconnection of external conditions and internal limiting factors.

Since the two older samples of green leaves give the same assimilation with different chlorophyll-content, it may be assumed that, under these experimental conditions, the photochemical stage is not limiting. Further evidence is supplied by the fact that other experiments of Willstätter show that normal green leaves at 25° C. reach their maximum assimilation with half the above light (about 24,000 lux). That the assimilation of the golden variety is limited by the photochemical stage is indicated by the fact that the assimilation, with the smaller chlorophyll-content, is less than that of the normal leaves, and that even at 48,000 lux the leaves show no signs of having reached their maximum of assimilation. Most probably, with still further increase of illumination and the temperature still 25° C., they would reach the same maximum as the green leaves, provided the chemical stage allowed of such activity.

It is interesting that, when through weak illumination, the photochemical stage is limiting in both the mature green and the golden leaves; the assimilation is not proportional to the chlorophyll-content, the ratio of the former to the latter being greater in the golden variety.

These facts may be expressed as follows in terms of Willstätter's assimilation number. With the temperature at 25° C. the assimilation number of both types of leaves increases with increase of illumination from zero to 24,000 lux, since the assimilation increases without change in the chlorophyll-content. Throughout this range the assimilation numbers of the golden leaves are much greater than those of the normal green leaves. On further increasing the illumination beyond 24,000 lux, the assimilation number of the green leaves remains constant, but that of the golden variety continues to increase until the maximum of assimilation is reached at a point where the chemical stage becomes limiting. Since, under all conditions of illumination, the assimilation numbers of the golden leaves are higher than those of the green, mere comparison of assimilation numbers gives no indication as to what stage or factor is limiting. Fuller consideration of illumination and chlorophyll-content such as that given above is necessary.

Willstätter comments upon the similarity of his etiolated leaves of small chlorophyll-content to the golden varieties, in that both have high assimilation numbers. This similarity is not surprising, since both types of leaves are more or less fully developed except for small chlorophyll-content, and since at 25° C. and 48,000 lux the chlorophyll in fully-greened leaves is greatly in excess of the amount required for full assimilatory activity. This similarity, however, does not warrant any conclusions such as those drawn by Willstätter as to the nature of the limiting factor or stage in his etiolated leaves.

In the case of Willstätter's etiolated leaves there are no results for material which is definitely comparable in protoplasmic development, as there are in the case of the mature green and the mature golden leaves considered above. In the earlier stages of development of leaves a difference in the age of a day may represent a considerable difference in the degree of protoplasmic development. Nor are there results for identical material under different conditions of illumination, as is the case in our experiments.

An analysis of Willstätter's results for young spring leaves gives definite evidence that chlorophyll is not here the factor limiting the assimilation. First, since in the youngest sample with a chlorophyll-content of 6·6 the assimilation is only 0·072, whereas in the golden variety the assimilation is

0·103 with a chlorophyll-content of only 1·9, the indications are against chlorophyll being limiting in the spring leaves. Secondly, these indications are borne out by the fact that young leaves 18 days older than the first sample exhibit an increase of assimilation of about 100 per cent. with an increase in chlorophyll-content of only 30 per cent. Here the assimilation number has increased with increase of chlorophyll-content; a comparison of mature golden and mature green leaves shows the opposite relation between assimilation number and chlorophyll-content. It is impossible to say in this case whether the limiting factor is involved in the photochemical or the chemical part of the mechanism. If in both cases of spring leaves the external limiting factor be temperature, as it is in the mature leaves, then the internal limiting factor would be concerned with the chemical part; but if, on the other hand, light be the external limiting factor, as it is in the leaves of the golden variety, then the internal factor would be concerned with the photochemical part.

It has thus been shown that there are three lines of evidence indicating that chlorophyll is not the limiting factor in the young stage of leaf development. In Irving's experiments the chlorophyll continued to increase, to a certain point, without any sign of assimilation; in the case of Willstätter's young leaves, as in the case of those of Irving, when they once reached the stage of beginning to assimilate, the assimilation increased out of proportion to the increase in chlorophyll; and thirdly, in the writer's experiments, the assimilation increased with no increase in chlorophyll.

Summary.

(1) The development of a leaf begins with a phase in which it has no photosynthetic power. The development of this power is shown to lag behind greening, so that a young green leaf may exhibit slight or zero photosynthesis. This means that photosynthetic activity demands the development of some internal factor other than chlorophyll. The photosynthetic potentiality of this factor rapidly increases with age, day by day, whether the leaf is in the light or in the darkness, and even though there is no concurrent increase in the amount of chlorophyll.

(2) It has been possible to demonstrate this by keeping the leaf when lighted for photosynthesis in an atmosphere of hydrogen with only a trace of oxygen, in which condition there is no increase in the initial amount of chlorophyll.

(3) The photosynthesis is measured in an atmosphere of hydrogen, to which carbon dioxide has been added. The partial pressure of the oxygen

produced is kept extremely low by being quickly removed and estimated by a new method due to Dr. F. F. Blackman.

(4) If a leaf is cut from a seedling (*Phaseolus*) in the dark at an early stage and partially greened by exposure to light in air, its photosynthetic activity will be very small or nothing, while if exactly the same procedure is repeated with a similar leaf from the same plant a few days later, quite strong photosynthetic activity will be found.

(5) This alteration of the internal factor with age explains why Willstätter has failed to confirm the observations published by Miss Irving in 1910, proving that young leaves of a full green colour might yet possess no power of photosynthesis. Miss Irving used very young leaves, while Willstätter happened to use leaves cut from plants some nine days older. In the present research the results of both observers have been obtained in succession on the same plant.

(6) The conception of the photosynthetic process as consisting of stages such as diffusion, photochemical, and chemical stages necessitates the consideration of external factors when stating that an internal factor is limiting.

(7) Consideration of the results here presented shows that the activity of the photochemical part of the photosynthetic mechanism in young seedling leaves as compared with that of more mature leaves is in some way limited, but apparently the photochemical part depends for its intensity not only upon chlorophyll but also upon some other factor. This factor increases with age during the early stages of leaf development.

(8) Willstätter's conclusion that chlorophyll was limiting in his results with etiolated leaves is based upon insufficient evidence. Full analysis of his results with young spring leaves shows that chlorophyll is not limiting, just as it is not limiting in Miss Irving's experiments, and in the experiments described in the present paper.

APPENDIX.—*Assimilation Experiments.*

Each Table refers to the experiment of that number in the text. The first measurement of assimilation was made immediately after greening, except in the "A" experiments, when the leaves were kept in the dark during the period between the time of greening and measurement of assimilation. During the periods between the estimations of assimilation the leaves were kept in the dark in air at the temperature of the laboratory (14°–15° C. in Experiments I–VI) except in Experiment IA when the temperature was 24°—25° C. A preliminary of about one hour in the apparatus, prior to estimation of assimilation, was allowed.

Tables I–VII.—*Phaseolus vulgaris.*

Date.	Temperature, C.	Distance of lamp from chamber in cm.	Oxygen per grm., dry-weight per hour, c.c.	Duration of estimations in hours.	History of material.
			Table I.		
17.x.19	14°–15°	6	1·2 1·3	5½	Sown 8.x.19, at 25° C. Cut at 9 P.M., 16.x.19, and exposed to lamp at 14°–15° C. for 13 h. Leaf colour, green-yellow.
18.x.19	14°–15·5°	6	2·4 2·7	3½	
20.x.19	15°	6	7·2 6·8	2¼	
21.x.19	15°	6	7·5 6·7	4	
			Table IA.		
18.x.19	15°	6	4·1	2¼	As in Table I.
19.x.19	13°	6	5·2 5·7 5·7	4¼	
20.x.19	15°	6	6·5 6·8	3	
			Table II.		
22.x.19	15°–16°	6	7·7 7·8	6	Sown 8.x.19, at 25° C. Cut at 7 P.M., 21.x.19, and exposed to lamp at 14°–15° C. for 15 h. Leaf colour, green-yellow.
23.x.19	15°–16°	6	8·5 10·0	5	
24.x.19	15°–16°	6	10·2 9·8	3	
			Table IIA.		
24.x.19	15°–16°	6	11·0 10·4	2½	As in Table II.
			Table III.		
4.x.19	12°–13°	6	0	7	Sown 26.ix.19, at 25° C. First six days from sowing at 25° C., remaining day at 14°–15° C. Cut at 10.45 A.M., 3.x.19, and exposed diffuse daylight for 8 h. and 17 h. lamp. Leaf-colour, yellow-green.
5.x.19	12°–13°	6	4·8 5·1	3	
5.x.19	12°–13°	12	3·3	3	
6.x.19	13°–14·5°	6	8·6 10·3 11·0	4	
6.x.19	13°–14·5°	12	4·3	2	

Tables I–VII—*continued.*

Date.	Temperature, C.	Distance of lamp from chamber in cm.	Oxygen per grm., dry-weight per hour, c.c.	Duration of estimations in hours.	History of material.
			Table IIIA.		
7.x.19	14°–15°	6	9·1 9·2	3	As in Table III.
7.x.19	14°–15°	12	3·9	1¾	
			Table IV.		
8.x.19	12·5°–14°	6	4·4 6·7 7·0	4½	Sown 26.ix.19, at 25° C. First six days from sowing at 25° C., remainder at 14°–15° C. Cut at 9.30 P.M., 7.x.19, and exposed to lamp for 13 h. Leaf colour, green-yellow.
8.x.19	12·5°–14°	12	3·3	3	
9.x.19	12°	6	7·6 7·9	3	
9.x.19	12°	12	3·7 3·8	2¼	
10.x.19	11°	6	8·7 9·6	3	
			Table IVA.		
10.x.19	11°	6	7·3	2	As in Table IV.
11.x.19	10·5°	6	7·0 7·0	4	
			Table V.		
15.x.19	14°	6	11·9 12·1	2	Sown 26.ix.19. Plant exposed to diffuse light on morning of 11.x.19, until 15.x.19. Leaves, normal green.
			Table VI.		
25.x.19	14·5°–15·5°	6	11·5 12·3	1½	Sown 8.x.19. Plant exposed to diffuse light, 19.x.19, until 25.x.19. Leaves, normal green.
25.x.19	14·5°–15·5°	12	5·0 5·0	3½	
			Table VII.		
21.vi.19	17·5°–18·5°	6	0	10½	Sown 14.vi.19. Exposed 19.vi.19, until 21.vi.19; diffuse light. Colour, grass green. Leaves dead at end of experiment.
22.vi.19	17°	6	0	1	

Tables VIII–Xa.—*Avena Sativa.*

Date.	Temperature, C.	Distance of lamp from chamber in cm.	Oxygen per grm., dry-weight per hour, c.c.	Duration of estimations in hours.	History of material.
Table VIII.					
19.vi.19	21°–23°	6	4·4 6·6	3½	Sown 12.vi.19, at 25° C. Exposed 18.vi.19, to diffuse daylight until grass green.
19.vi.19	21°–23°	12·5	2·8	3	
19.vi.19	21°–23°	6	14	2	
20.vi.19	20°	6	23	1	
Table IX.					
23.vi.19	18°	12·5	1·4 1·7 2·2	2	Sown 18.vi.19, at 25° C. Grown in diffuse daylight. Colour, grass green.
23.vi.19	18°	6	4·9 7·1 10·0	6	
Table X.					
30.vi.19	15°–17·5°	6	2·4 3·8	8½	Sown 23.vi.19, at 25° C. Exposed 29.vi.19, for 7 h. Colour, green-yellow.
1.vii.19	17·5°–19·5°	6	6·1 7·0	2½	
Table Xa.					
1.vii.19	20°–25°	6	5·2 6·1	1½	As in Table X.
2.vii.19	20°–25°	6	5·8	2¾	

Table XI.—*Vicia Faba.*

Date.	Temperature, C.	Distance of lamp from chamber in cm.	Oxygen per grm., dry-weight per hour, c.c.	Duration of estimations in hours.	History of material.
8.viii.19	19°–22·5°	6	0 0·9	7½	Sown 31.vii.19. Exposed to diffuse daylight, 6.15 P.M., 7.viii.19, to 10.15 A.M., 8.viii.19. Colour, yellow-green.
9.viii.19	22°–25°	6	1·1 1·6	9	

Respiration Experiment.

Table XII.—*Phaseolus.*

Date.	Temperature, C.	CO per grm., dry weight per hour, c.c.	Remarks.
4.xi.19	11°–12°	1·40	Sown 29.x.19. Dry weight, 0·14 grm. Fresh weight, 0·74 grm.
5.xi.19	11°–12°	0·85	
6.xi.19	10°–11°	0·77	

Studies on Synapsis. I.—Oogenesis in the Hymenoptera.

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[Plates 4–9.]

For establishing a correlation between genetic and cytological phenomena, the Hymenoptera furnish a series of forms which offer fertile possibilities; the production of males from eggs which segment without fertilisation makes it possible to state definitely the point in the germ cycle at which the determination of sex is effected. While, however, much work has been done from the cytological standpoint in connection with the group, our knowledge is still inadequate with respect to those species which produce females as well as males by parthenogenesis. What little is known of this subject has been derived exclusively from a study of the polar and cleavage mitoses; and in view of the importance of treating the germ cell cycle as a whole, and also of the unfavourable material which heavily-yolked ova necessarily afford, it seemed desirable to investigate oogenesis in typical and completely agamic Hymenoptera, more especially because data relating to the maturation prophases of the female germ cells in insects are much less numerous than in the case of spermatogenesis. The original purpose of the author in pursuing the present investigation arose out of previous work on nuclear phenomena in the late ovarian oocytes of *Neuroterus,* which yielded indications of post synaptic syndesis. Agar has described a second conjugation of chromosomes after a temporary dissociation of univalents in *Lepidosiren,* unconfirmed hitherto in any other animals; and since the